Thermal behaviour and biological activity of pyrethroids in smoke-generating formulations

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Abstract: Smoke-generating insecticide formulations are nowadays very important for the control of Chagas' disease vectors.

In this work, the thermal decomposition of different pyrethroid insecticides in smoke-generating formulations has been studied. Their recovery from the smoke has been determined, and the effect on this of the addition of foaming agents such as cyanoguanidine (CNG) or azodicarbonamide (ADC), or antioxidant agents such as butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) has been assessed. In each case the best smoke-generating formulation was established. Calorimetric studies were done to justify the behaviour of the smoke-generating mixtures.

The isomerization process for different pyrethroids was also studied, to establish the influence of the foaming agents in inhibiting isomerization to less active pyrethroids.

Smoke-generating mixtures containing β-cypermethrin and *cis*-permethrin as insecticides and CNG or ADC as foaming agents were evaluated for their insecticidal activity against nymphs I of *Triatoma infestans*, vector of Chagas' disease. The best effect was found with β-cypermethrin formulated with ADC, with LT_{50} values lower than 5 min.

Keywords: pyrethroids; smoke generators; thermal behaviour; *Triatoma infestans*

1 INTRODUCTION

Smoke-generating insecticide formulations nowadays are important tools for the control of Chagas' disease vectors, as a result of previous work of our laboratory.[1–4]

Pyrethroids such as permethrin and cypermethrin have been widely used in this type of formulation. Japanese patents have reported the use of foaming agents (eg CNG and ADC) to improve the thermal stability of pyrethroids in smoke-generating mixtures, but no details were given about the protection process.[5,6] Thus, it is important to consider the thermal decomposition processes of pyrethroids produced by the combustion of typical smoke-generating mixtures. A better knowledge of these processes in smoke-generating formulations is necessary to improve the delivery efficiency of active ingredients and to ensure safe usage.

Photochemical decomposition of pyrethroids has been studied in a variety of systems, but not too much information is available about the thermal behaviour of these insecticides.[7] There have been studies of the pyrolysis of pyrethrins, allethrin, propathrin and furamethrin related to the widespread use of mosquito coils and electrically heated mats at temperatures around 120–180 °C.[8–11]

The present report deals with the recovery of pyrethroids from the smoke delivered from smoke-generating formulations. The influence of foaming agents and antioxidants on their thermal decomposition was studied as well as the calorimetric properties of the mixtures. Also, the isomerization processes during pyrethroid delivery were evaluated. The fumes delivered by smoke-generating formulations with the best recovery and least isomerization were assayed for their insecticidal effect on nymph I of *Triatoma infestans* Klug.

2 MATERIALS AND METHODS

2.1 Insecticides

cis-Permethrin (*cis*:*trans* 99:1) was obtained in our laboratory according to patent P 960105372 (CIPEIN-1996) from permethrin 97.4% (*cis*:*trans* 45:55), Chemotecnica (Argentine); *trans*-permethrin 98.1% (*cis*:*trans* 3:97), cypermethrin 90% (*cis*:*trans* 40:60) and β-cypermethrin 95.1% (*cis*:*trans* 60:30), were provided by Chemotecnica (Argentine). Tetramethrin

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(94%) and phenothrin (93.4%) were obtained from Sumitomo (Japan).

2.2 Chemicals

Potassium chlorate, technical grade was from Parafarm (Argentine), dextrin was from Aldrich (USA), kaolin (aluminium silicate), technical grade Serain Juarez (Argentine), azodicarbonamide (ADC) 97%, and cyanoguanidine (dicyandiamide) 99%, were from Aldrich (USA). Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were from Sigma. All solvents used were analytical grade and distilled when necessary.

2.3 Biological material

First-instar nymphs of *Triatoma infestans* were obtained from a colony reared in our laboratory at 26–28 °C and photoperiod of 12:12 h.[12] The experimental work was done on five-day-old nymphs I, starved since hatching (12 ($\pm$0.2) mg weight).

2.4 Smoke-generating mixtures

A basic smoke-generating mixture composed of potassium chlorate, +dextrin+kaolin (16+10+74 by weight) was ground in a small coffee grinder. To this mixture solid pyrethroid (15 g kg^{-1}) and various amounts of ADC and CNG were added by mechanical agitation. When antioxidants, liquids or low-melting-point insecticides were used, these components were dispersed in dichloromethane and the solvent evaporated under vacuum.

2.5 Pyrethroid decomposition and isomerization during combustion

A 1-g tablet of smoke-generating mixture was obtained by using a manual pellet press (Parr Instrumental Co, Illinois, USA). Tablets were burned in a modified combustion flask (Thomas Schoeniger flask, USA) to which a side tube with a Teflon stopper was added to compensate for pressure changes. Tablet ignition was performed with an IR lamp (GE Projection Lamp, USA). After combustion the flask was cooled in an ice bath and washed quantitatively with dichloromethane. The volume of dichloromethane was then made up to 10 ml and analysed by chromatographic techniques to determine the amount of pyrethroid delivered in the smoke. In the case of permethrin and β-cypermethrin the concentration of each geometric isomer was also measured.

2.6 Chromatographic analysis

2.6.1 GLC

Gas liquid chromatography was used to analyse all pyrethroids except β-cypermethrin. A Shimadzu 6AM Gas Chromatograph (Japan) with 2.5 m $\times$ 2 mm glass column, QF-1 (trifluorpropylmethyl silicone, Supelco, USA), and FID detector, column temperature 210–220 °C (according to the pyrethroid analysed), injector and detector temperature 250 °C was used. Calibration was performed with standard solutions of the respective pyrethroids. This technique was used because it provides better separation of *cis-trans* pyrethroid isomers.[13]

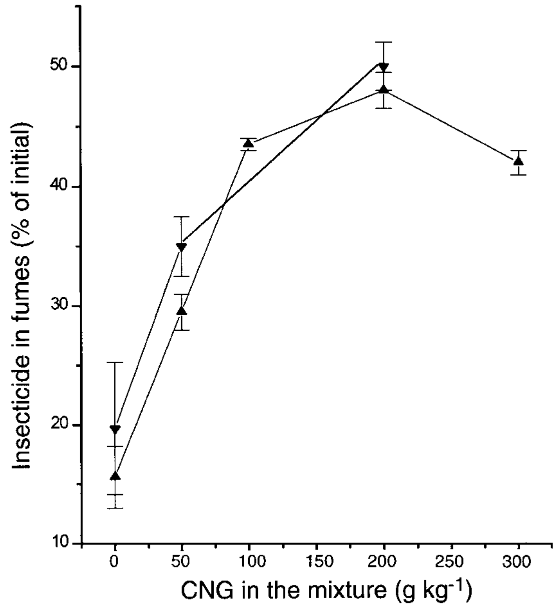

Figure 1. Quantitative recovery of ▼ *cis*-permethrin and ▲ permethrin from smoke from mixtures containing increasing amounts of CNG.

2.6.2 HPLC

High performance liquid chromatography: β-cypermethrin analysis was done in a Jasco Familic 300S, with a chiral Pirkle column (*R*- *N*- (3,5-dinitrobenzyl-phenylglycine), 4.6 mm $\times$ 250 mm and hexane + isopropanol (99.9 + 0.1 by volume) as eluent at 1 ml min^{-1} and UV detector UVIDEC-100-VI at 254 nm.[14]

2.7 Non-volatilized insecticide (residue)

Tablets of insecticide were burned under air extraction conditions in order to avoid deposition of the smoke. The black residue obtained was extracted and analysed by GLC or HPLC according to the pyrethroid assayed.

2.8 Differential scanning calorimetry and thermogravimethric analysis

A Dupont 900 Thermal Analyser (USA) was used, with a temperature program 20 °C min^{-1} and an empty capsule as reference. The nitrogen flux was 50 ml min^{-1}.

2.9 Insecticidal activity

Groups of 10 nymphs I of *Triatoma infestans* were exposed to the insecticide smoke in an 0.7-m cubic glass chamber, the front panel of which had a tightly fitting door and four holes, each 5 cm diameter, located 15 cm from the roof. The nymphs were confined in cylindrical plastic jars (5 cm high, 5 cm diameter) covered at the open top with nylon gauze.

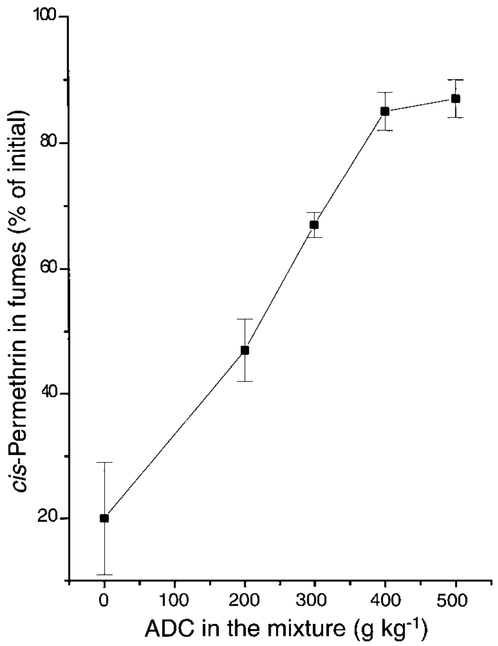

Figure 2. Quantitative recovery of permethrin in smoke from mixtures containing increasing amounts of ADC.

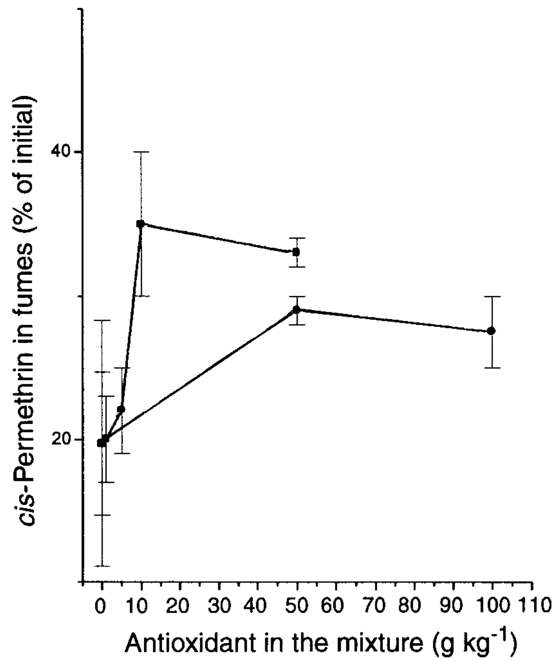

Figure 3. Quantitative recovery of permethrin from smoke from mixtures containing increasing amounts of (■) BHT and (●) BHA.

The jars were inserted in the front panel holes with the gauge-covered tops inside the chamber. A 300-mg tablet containing $30\,g\,kg^{-1}$ of *cis*-permethrin or *β*-cypermethrin was fixed in the centre of the chamber and burned. The exposure time was taken from the moment of ignition of the tablet. At 5, 10, 20 or 30 min, the exposed insects were transferred to clean jars fitted with filter paper. Control groups of nymphs I were exposed to a smoke-generating mixture without insecticide. Each experiment was replicated three times.

Mortality was assessed 24 h after treatment to determine the LT_{50} value (time for 50% mortality) using Probit analysis (EPA probit used for calculating EC values v.1.4).[15]

3 RESULTS AND DISCUSSION

3.1 Pyrethroid decomposition during combustion

Smoke-generating mixtures with permethrin or *cis*-permethrin as the active component, and containing increasing amounts of CNG and ADC were evaluated (Figs 1 and 2).

An increased recovery of pyrethroid was observed in the smoke when the concentration of the foaming agent was increased. As can be seen in Fig 1, an optimal recovery was achieved for permethrin and *cis*-permethrin for mixtures containing $200\,g\,kg^{-1}$ CNG. Mixtures with *cis*-permethrin and $300\,g\,kg^{-1}$ CNG. did not have satisfactory combustion properties.

With ADC as foaming agent, as can be seen in Fig 2, the insecticide recovery reached a maximum for concentrations greater than $400\,g\,kg^{-1}$. With concentrations over $500\,g\,kg^{-1}$ ADC, tablet combustion failed.

Thus, mixtures containing $200\,g\,kg^{-1}$ CNG or $400\,g\,kg^{-1}$ ADC, were evaluated with different pyrethroids (Table 1). In all cases an increase in pyrethroid recovery from the smoke was observed. Recoveries obtained with tetramethin, permethrin, cypermethrin and phenothrin ranged from 15 to 60% with the addition of CNG or ADC.

The incorporation of antioxidants such as BHT or BHA to the smoke-generating mixtures at concentrations under $50\,g\,kg^{-1}$ (Fig 3) did not show a significant increase in permethrin recovery.

To study the influence of the initial concentration of the insecticide on its delivery in the smoke, mixtures containing $200\,g\,kg^{-1}$ CNG or $400\,g\,kg^{-1}$ ADC as

Table 1. Quantitative recovery of pyrethroids in fumes from mixtures containing ADC and CNG as foaming agents

	Insecticide recovery in the smoke (% of initial)		
Insecticide[a]	*No foaming agent*	*200 g kg⁻¹ CNG*	*400 g kg⁻¹ ADC*
Tetramethrin	0.5 (±0.5)	15.0 (±2.5)	63.0 (±2.0)
Phenothrin	22.0 (±4.0)	37.6 (±6.0)	–
Cypermethrin	17.0 (±1.5)	42.0 (±1.0)	–
β-cypermethrin	17.0 (±1.5)	48.5 (±1.5)	43.5 (±2.0)

[a] The initial concentration of the insecticide in the tablet was $15\,g\,kg^{-1}$.

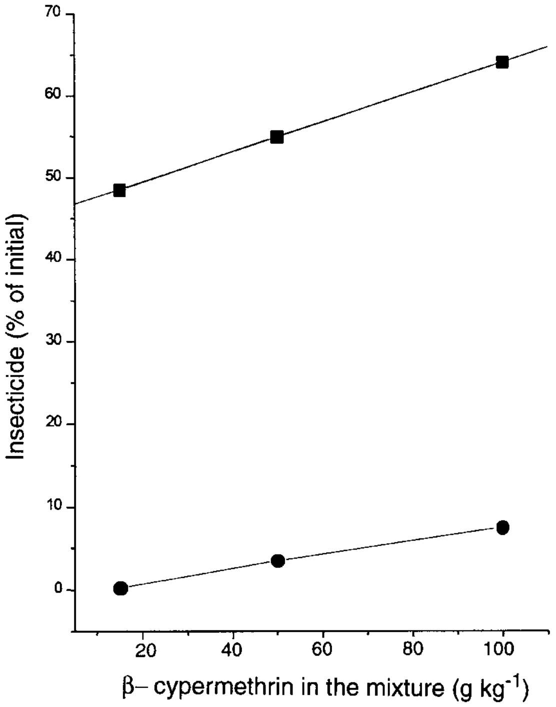

Figure 4. Quantitative recovery of β-cypermethrin (■) from smoke and (●) non-volatilized insecticide, from mixtures containing 200 g kg^{-1} CNG.

foaming agents and variable amounts of β-cypermethrin as active ingredient were evaluated. The recovery in the smoke was measured with respect to the initial content of insecticide (Figs 4 and 5).

3.2 Non-volatilized insecticide

The non-volatilized insecticide in the burned residue was quantified and is shown in Figs 4 and 5. The

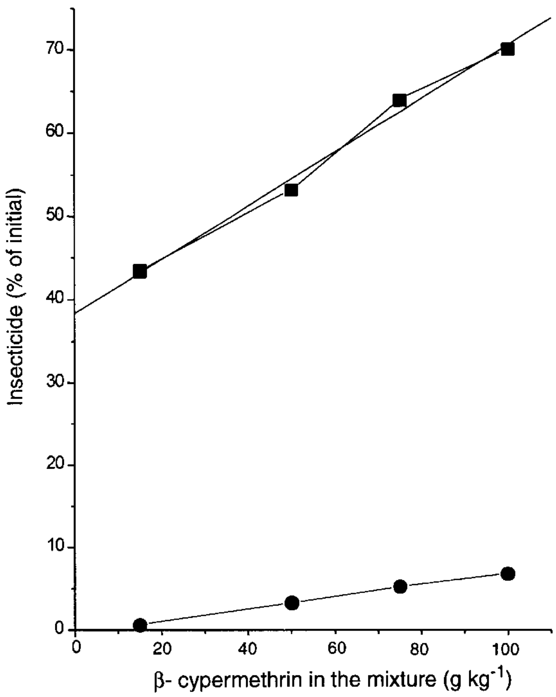

Figure 5. Quantitative recovery of β-cypermethrin (■) from smoke and (●) non-volatilized insecticide from mixtures containing 400 g kg^{-1} ADC.

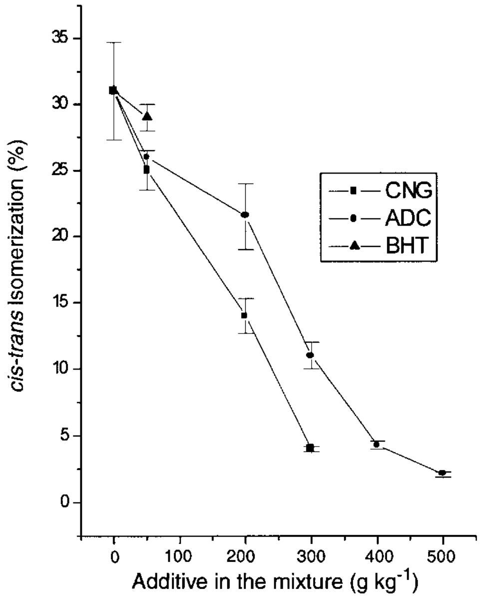

Figure 6. Influence of foaming agents and antioxidant concentrations on *cis–trans* isomerization of *cis*-permethrin. Isomerization is measured as (% final *trans*/% initial *cis*) × 100. Each additive was incorporated into the mixture in the maximum concentration compatible with combustion.

percentage recovery of the insecticide increased and also the non-volatilized residue.

Because the combustion heat (ΔH) of the smoke-generating mixture is constant, an increase in the percentage of insecticide in the formulation reduces the quantity of heat absorbed per mass unit of insecticide. In such a case the vaporization and degradation process of pyrethroids could be reduced with a consequent increase of insecticide concentration in the non-volatile residue and the smokes.

3.3 Pyrethroid isomerization during combustion

The different isomer content of pyrethroid is strongly related to biological activity. Permethrin has four isomers: (1*R trans*, 1*R cis*, 1*S trans* and 1*S cis*) that present different toxicities to insects.[16]

On the other hand, β-cypermethrin is a third-generation pyrethroid, enriched in the active isomers of cypermethrin: the pairs *cis* II (1*S cis* α *R*, 1*R cis* α *S*) and *trans* II (1*S trans* α *R*, 1*R trans* α *S*). This mixture is particularly active against Chagas' disease vectors.[17]

As a general trend, *cis* isomers of permethrin are 2–4 times more toxic to insects than the *trans* isomers. For

Table 2. Isomerization of permethrin during combustion of smoke-generating mixtures with and without CNG

Isomeric ratio before combustion (cis:trans)	*Isomeric ratio after combustion (cis:trans)* With CNG[a]	Without CNG
99:1	85:15	66:33
45:55	40:60	40:60
3:97	5:95	20:80

[a] 200 g kg^{-1} CNG.

Table 3. Influence of incorporation of foaming agent CNG on *cis-trans* isomerization of β-cypermethrin during combustion

CNG ($g\,kg^{-1}$)	*Inactive isomers (%)*[a]
0	34.2
200	19.5

[a] *cis* I + *trans* I (inactives) as percentage of total. Initial proportion of inactive isomers was 2.0%.

Table 4. Influence of β-cypermethrin content on *cis-trans* isomerization during combustion

β-Cypermethrin in smoke generator (%)	*Inactive isomers after combustion (%)*[a]
1.5	17.2
5	12.5
10	8.3

[a] As Table 3.

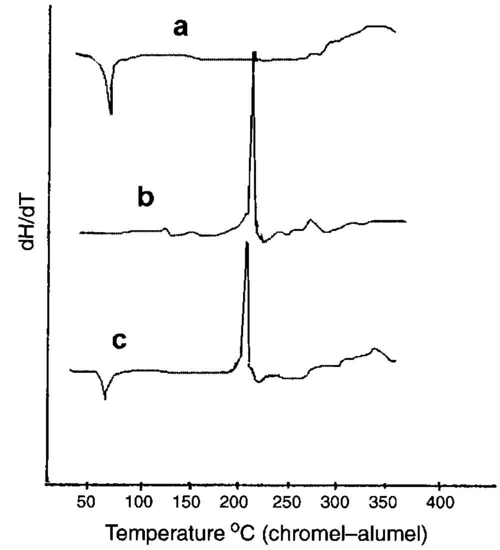

Figure 8. DSC curves of (a) β-cypermethrin, (b) smoke-generating mixture with ADC and (c) smoke-generating mixture with β-cypermethrin and ADC.

T infestans, a Chagas' disease vector, this difference of toxicity between isomers varies with nymphal stage.[18]

Cis–trans isomerization occurs during the combustion of the smoke-generating mixture and, as can be seen in Fig 6, the addition of foaming agents such as CNG or ADC partially inhibited the isomerization process. In the case of permethrin, the percentage of isomerization to *trans* isomer was diminished. The addition of an antioxidant did not produce a significant change in the isomerization process. It can be seen in Table 2 that the percentage transformation also depends on the initial isomer ratio. As a general trend it was observed that isomerization processes tend to *cis-trans* equilibrium.

β-Cypermethrin, is an isomeric mixture enriched in *cis* II and *trans* II pairs, but on combustion the inactive pairs *cis* I and *trans* I are formed in the fumes, although the process is partially inhibited by foaming agents (Table 3). In addition, increasing insecticide concentration also results in proportionally less isomerization (Table 4).

3.4 Calorimetric studies

These studies are usually performed in order to determine the inflammability temperature of the mixtures and the compatibility of its components and so ensure safe storage.

Differential scanning calorimetry and thermogravimetric curves of three insecticides (*cis*-permethrin, β-

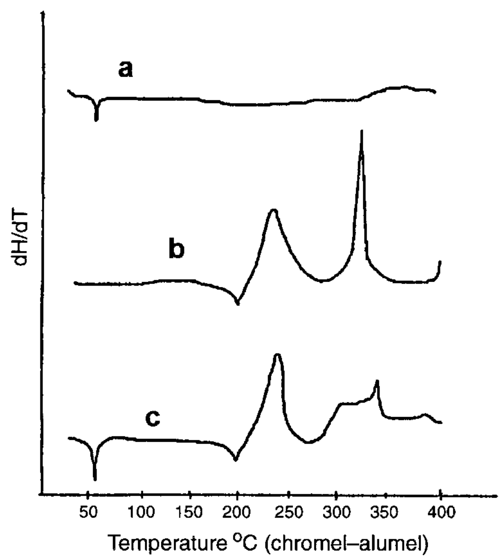

Figure 7. DSC curves of (a) *cis*-permethrin, (b) smoke-generating mixture with CNG and (c) smoke-generating mixture with *cis*-permethrin and CNG.

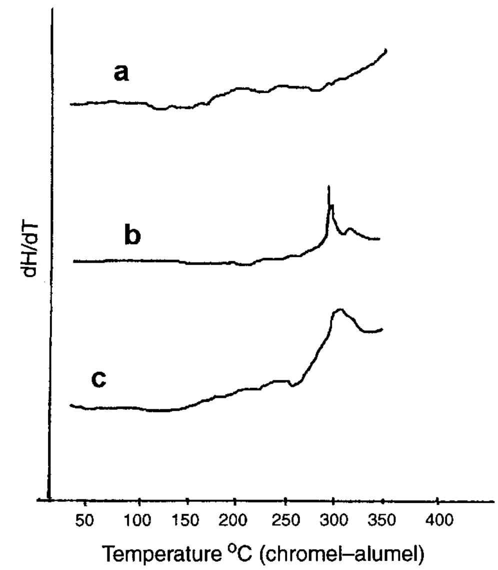

Figure 9. DSC curves of (a) phenothrin, (b) smoke-generating mixture without foaming agent, (c) smoke-generating mixture with phenothrin.

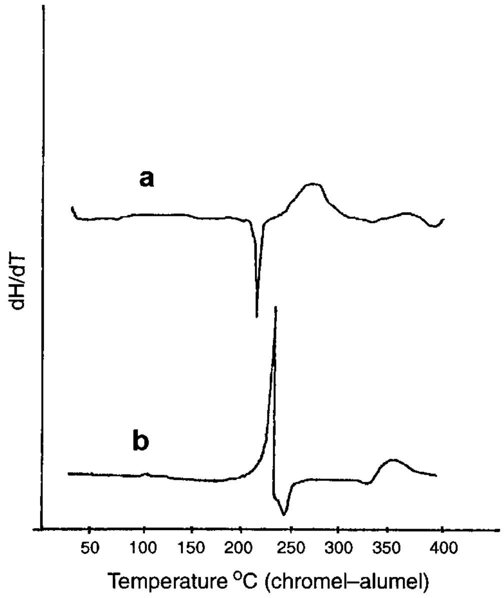

Figure 10. DSC curves of (a) pure CNG and (b) pure ADC.

cypermethrin and phenothrin), CNG, ADC and smoke-generating mixtures without foaming agent and with the addition of CNG or ADC were performed.

3.4.1 Insecticides, CNG and ADC

The thermogram of *cis*-permethrin (Fig 7) showed that fusion occurs at 60 °C (endothermic) and its decomposition starts at 310 °C; *β*-cypermethrin (Fig 8) fuses at 70 °C and its decomposition starts at 285 °C and phenothrin, which exists as a liquid at room temperature, starts to vaporize and decompose at 180 °C (Fig 9).

The thermogram of CNG (Fig 10) shows an endothermic fusion peak at 212 °C and an exothermic decomposition peak at 232 °C, while ADC decomposes without previous fusion at 225 °C (Fig 10) and this process is followed by an endothermic peak owing to a change in the solid product.[19] Exothermic peaks are attributed to gas formation, during which insecticides are carried out of the mixture.

3.4.2 Smoke-generating mixtures

When the smoke-generating mixtures without pyrethroid compounds were assayed the ignition started at 330 °C (Fig 9). For the smoke-generating mixtures containing 200 $g\,kg^{-1}$ CNG or 400 $g\,kg^{-1}$ ADC decomposition occured in two independent (flame temperature) steps, the first one corresponding to the foaming agent (213 °C and 225 °C respectively) itself and the second to the rest of the mixture (Figs 7 and 8 respectively). The decomposition temperature of the mixtures did not decrease with the incorporation of insecticides (Figs 7–9). The melting of the insecticides occured before and independently of the thermal behaviour of the rest of the mixture. As can be observed from Figs 7–9, mixtures containing foaming agents produced an earlier gas liberation at lower temperatures than the decomposition of the insecticides, considerably increasing their recovery from the smoke. In contrast, when there was no foaming agent present, the mixtures flamed at temperatures closer to or higher than the insecticide decomposition temperatures.

3.5 Insecticidal activity

Mixtures containing *β*-cypermethrin and *cis*-permethrin as active ingredients and CNG or ADC in their optimal concentrations as foaming agents were evaluated for triatomicidal activity against nymphs I of *T infestans*. Table 5 shows that *β*-cypermethrin plus ADC or CNG has better activity than *cis*-permethrin plus ADC or CNG.

Thus, new smoke-generating canisters were developed for the control of Chagas' disease vectors, with lower ambient impact by the use of the active isomers of cypermethrin at lower concentration because of its higher recovery after delivery in the smoke.

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Table 5. LT_{50} values for *Triatome infestans* nymphs I exposed to insecticidal smokes.[a]

Insecticide ($g\,kg^{-1}$)	*Foaming agent*	LT_{50} *(min)*	*Confidence interval (min)*
cis-Permethrin(30)	ADC	8.03	4.07–11.68
	CNG	16.48	12.07–22.06
β-Cypermethrin(30)	ADC	<5	–
	CNG	<5	–

[a] 300 mg mixture were burned.

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